FINAL REPORT

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MOLECULAR GENETIC TECHNIQUES FOR SELECTION OF PRODUCTION TRAITS IN CATTLE

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Principal Investigators: F.C. Buchanan, A. Van Kessel
Project title: Molecular genetic techniques for selection of production traits in cattle (Obese gene regulation of appetite and fat deposition in cattle).

a) Summary

This project focused on the genetics and physiology of leptin, the hormonal product of the obese gene, in control of fat deposition in cattle. Carolyn Fitzsimmons and Sheila Schmutz of the Department of Animal and Poultry Science, University of Saskatchewan identified a single nucleotide polymorphism (SNP) in the nucleotide (DNA) sequence of leptin. The SNP was a cytosine (C) to thymine (T) transition resulting in the substitution of a cysteine for an arginine at position 4 of the mature leptin peptide. Presence of the T allele (cysteine at position 4) was associated with increased carcass fat content at slaughter in beef bulls. A primary objective of this project was to confirm a relationship between the leptin SNP and the rate of fat deposition in steers. We also wanted to determine if the SNP was directly responsible for physiological changes leading to altered rates of fat deposition. As such the SNP would be designated a causative SNP and its power for genetic selection significantly enhanced.

In a study involving 96 head of Angus, Hereford and Charolais crossbred steers, finished to either 6 or 12 mm of backfat, we have confirmed a relationship between the leptin SNP and rate of fat deposition. Cattle homozygous for thymine (TT) reached target backfat depths 14 days sooner than their homozygous cytosine (CC) counterparts and were 8% lighter in body weight (Van Kessel et al. 1999). Serum insulin and leptin concentrations were measured in the cattle prior to slaughter. Insulin is a primary endocrine regulator of leptin secretion, however, no effect of the leptin SNP on serum insulin concentration was observed. Serum leptin was measured through a collaboration with Dr. Yves Boisclair at Cornell University. A 3-way interaction among breed, backfat depth and leptin genotype was observed for serum leptin concentration. A significant effect of genotype was observed in the 12 mm Charolais crossbred group only, such that homozygous TT Charolais crossbreds had significantly higher plasma leptin versus homozygous CC Charolais crossbreds. Serum leptin concentration was higher, but not significantly, in TT Angus crossbreds at both backfat depths and in TT Hereford crossbreds in the 6 mm backfat group. The results suggested, although not strongly, that leptin genotype could impact leptin expression levels supporting the identified polymorphism as a causative SNP.

Leptin is expressed primarily in fat tissue. To quantify leptin expression levels in the fat tissue of cattle we developed and validated a bovine leptin ribonuclease protection assay (RPA). The assay was used to assess the impact of the leptin polymorphism on gene expression. Leptin expression was quantified in three separate groups of cattle selected based on leptin genotype (Buchanan et al. 2002). In each group of cattle, animals homozygous for thymine (TT) had a greater abundance of leptin mRNA in fat than their homozygous cytosine (CC) counterparts. The result supported the hypothesis that substitution of the arginine residue for cysteine results in a partial loss in the biological function of leptin. Homozygous TT cattle expressing leptin with reduced biological function would attempt to compensate by increasing the amount secreted. This compensation is not completely successful, however, and these cattle present with increased rates of fat deposition. The results of the leptin mRNA analysis and this hypothesis are consistent with the suggestion that the C to T transition discovered by C. Fitzsimmons and S.M. Schmutz is a causative SNP.

To further examine whether the leptin SNP is causative, we conducted a second study using 30 purebred unrelated Simmental steers selected based on leptin genotype. These animals were challenged with a period of feed restriction to monitor the effects of this metabolic perturbation on leptin secretion and to determine if genotype at the leptin locus affects the response. Serum leptin and insulin analysis demonstrated a significant reduction in the circulating concentration of both hormones with feed restriction. Leptin genotype, however, did not significantly influence the serum insulin or leptin profile. However, cattle having at least one T allele showed a tendency for higher serum leptin levels.

A primary objective of this research was to confirm that the identified leptin SNP is a causative SNP, responsible for significant differences in appetite and/or rate of fat deposition ultimately affecting carcass quality and/or cost of production. This information is critical to positioning the leptin SNP as a reliable commercial selection marker. Carcass and production data generated in the Angus/Hereford/Charolais study support the hypothesis that the leptin polymorphism is associated with carcass fat content. The leptin mRNA abundance data showing higher leptin expression in homozygous T cattle supports the hypothesis that the mutation is causative. Serum leptin data are less convincing with no clear effect of genotype on leptin concentration observed. At best, a trend to higher leptin levels in cattle carrying one or two copies of the T allele can be noted.

To provide definitive evidence that the leptin mutation is a causative mutation we set out to show that the leptin hormone containing cysteine at position 4 (corresponding to the T allele) has reduced biological function relative to the wild type (C allele) counterpart. Biological activity is often performed using *in vitro* bioassays, however, no bioassay of leptin activity is available. We therefore proposed to determine the relative biological

potency of the leptin forms using an *in vivo* sheep bioassay. Technical difficulties prevented the completion of this assay within the timeframe allotted. As a result, we have been unable to confirm a causative role of the SNP in altering fat deposition rate.

We have, however, confirmed an association between a polymorphism in leptin and fat deposition in cattle. In addition, we have provided evidence supporting the hypothesis that this is a causative mutation. Unfortunately, until biological activity of the two leptin forms can be assessed we cannot make a definitive claim as to the causative nature of the polymorphism. The project has provided sufficient new information to support the further evaluation of the leptin polymorphism as a sorting tool in commercial feedlots. Plans are currently progressing to test the economic significance of the SNP in large scale feedlot studies funding through industry involvement.

b) Technical Report

Background and objectives

The obese gene encodes a protein hormone called leptin which is secreted primarily by adipose tissue. Evidence indicates leptin regulates total body fat content by suppressing appetite and modifying nutrient partitioning. Genetic variation at this locus may account for either the generation of nonfunctional forms of the leptin hormone or varying concentrations. Dr. Sheila Schmutz and Carrie Fitzsimmons have found a non conservative single nucleotide polymorphism (SNP) in the obese gene that alters the amino acid sequence of the hormone and could alter the conformation. Hence the SNP could affect the biological activity of this protein hormone. Regression analysis using 156 Angus, Hereford, Simmental and Charolais bulls has shown animal homozygous for the T allele could have up to 2 mm more backfat than their homozygous C counterparts (Fitzsimmons M.Sc. Thesis, 1999).

In livestock species very little information is available regarding the role of leptin in control of fat deposition or appetite. The findings of Fitzsimmons and Schmutz support the contention that this hormone significantly impacts carcass composition and/or cost of production in cattle. The objectives of this project include:

1. Confirmation of an economically significant relationship between a single nucleotide polymorphism (SNP) in leptin and the propensity of steers to increase rate of fat deposition.
2. Development of analytical methods for measurement of bovine leptin gene and protein expression.
3. Classification of the leptin SNP as linked or causative with respect to the observed phenotype (increased rate of fat deposition).
4. Elucidation of the role of leptin in control of appetite and fat deposition in cattle.

Confirmation of the relationship between the leptin SNP and fat deposition and carcass quality in cattle will have significant positive impact on cattle production by targeting two separate markets. Livestock producers will be able to select bulls based on a test for the leptin SNP and the level of fat deposition desired in offspring. In addition, feedlot owners could potentially use the leptin SNP to segregate cattle based on propensity for fat deposition allowing optimization of feeding and marketing strategy to maximize profitability and/or carcass quality.

To accomplish our objectives we developed assays to measure expression of bovine leptin in cattle. To provide the tools for bovine leptin assay development, bovine leptin was cloned, expressed in E. coli and used to develop antibodies. We developed a ribonuclease protection assay to measure bovine leptin mRNA levels and collaborated with scientists as Cornell University to obtain access to a bovine leptin radioimmunoassay for measurement of serum leptin levels. These analysis tools were used to examine the physiological role of leptin in cattle as well as determining whether the leptin SNP affects leptin bioactivity. Of significant interest was to confirm whether the bovine leptin SNP is directly responsible for the observed changes in rate of fat deposition or is simply linked to a nearby gene which causes the change in phenotype. Usefulness and reliability of the SNP as a genetic selection tool would be markedly improved if a causative association was demonstrated.

Project Activities, results and conclusion

A. Development of leptin quantitative assay

Leptin mRNA quantification in fat

A ribonuclease protection assay (RPA) method of quantifying mRNA was developed. We multiplexed probes for bovine leptin mRNA with a bovine GAPDH probe as an internal relative control which is constitutively expressed (Figure 1). Essentially an antisense ^{32}P-labelled RNA probe was produced for both leptin and GAPDH. These were hybridized to total RNA extracted from fat samples and the unhybridized RNA is degraded using RNase leaving only the protected obese and GAPDH fragments to run on a polyacrylamide gel. Figure 1 is a scan of an autoradiograph showing protected fragments GAPDH and bovine leptin mRNA isolated from bovine adipose tissue. The density of the bands is proportional to the amount of specific mRNA in the tissue. To control for possible differences among samples in the amount of RNA used in the assay, the density of the leptin mRNA band was expressed as a percentage of the density of the constituitively expressed GAPDH band. Figure 1 shows an expected dose response increase in leptin and GAPDH mRNA with increasing amounts of total RNA (5, 10 and 20 ug) applied to the assay. Also, best results were obtained when a 1/50 dilution of RNase T1 was used.

One objective of the project was to determine whether the leptin SNP affected the biological activity of leptin and as such, whether the SNP was causative for the observed phenotype (increased fat deposition). A change in leptin expression levels among cattle having the C or T allele at the leptin SNP would be suggestive of an impact of the SNP on leptin biological activity. To investigate this we collected adipose tissue at slaughter from three independent groups of cattle. Group 1 consisted of 34 crossbred steers homozygous for the C allele (CC, n=17) or homozygous for the T allele (TT, n=17). Group 2 consisted of 15 animals selected from the Canadian Beef Reference Herd (8 TT and 7 CC). Group 3 consisted of 16 unrelated purebred Simmental steers (8 TT and 8 CC) used in the feed restriction study reported below. RPA analysis of leptin mRNA abundance in adipose tissue indicated an increase in leptin mRNA content in homozygous TT cattle that was significant for Groups 1 and 3 (Figure 2). Homozygous TT cattle in group 3 (Canadian Beef Reference Herd) had high leptin mRNA, but not significantly so.

The leptin mRNA abundance data was reported in the scientific journal, Molecular Genetics Evolution (Buchanan et al. 2002). The paper describes the leptin SNP identified by Fitzsimmons and Schmutz, including data from a previously completed study using 156 beef bulls showing the initial association between the SNP and carcass fat content. Inclusion of the leptin mRNA abundance data provided the first evidence of a causative relationship between the leptin SNP and rate of fat deposition. We hypothesized that the T allele of the leptin SNP, resulting in leptin with a cysteine at position 4 of the mature hormone resulted in a conformational change in the hormone causing a partial loss of

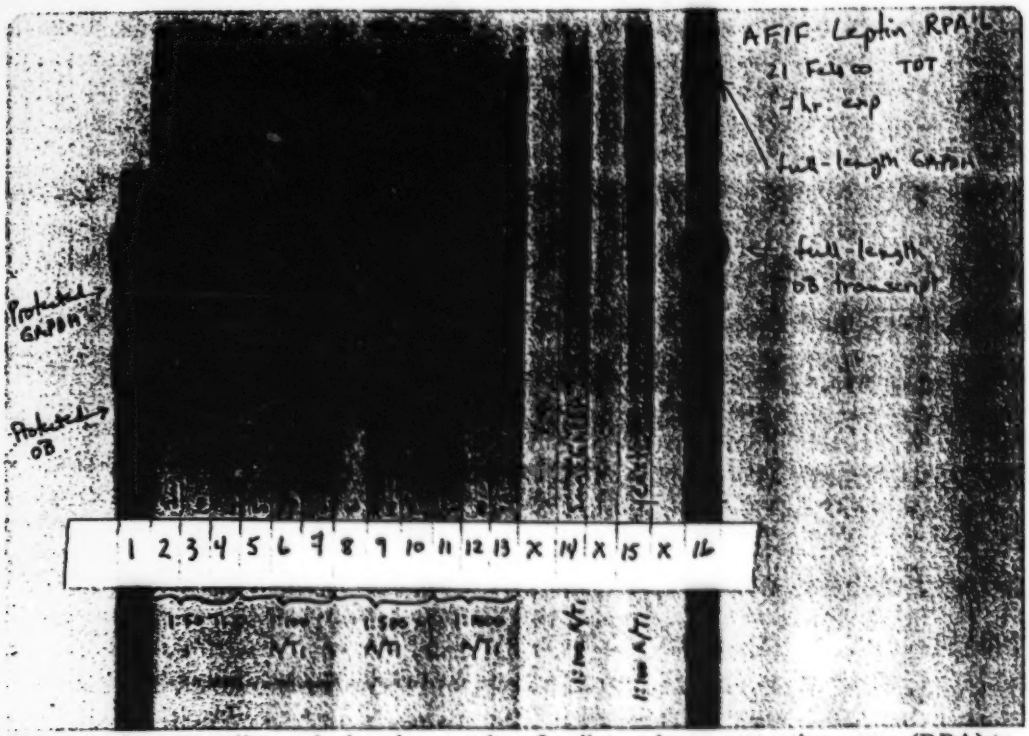


Figure 1. An autoradiograph showing results of a ribonuclease protection assay (RPA) to quantify leptin mRNA (Protected OB) relative to a constitutively expressed house keeping gene, glyceraldehydes phosphate dehydrogenase (protected GAPDH). A bovine total RNA pool extracted from fat tissue was used in the RPA at 5, 10 and 20 ug. Density of the leptin and GAPDH protected fragments is proportional to the amount of specific mRNA in the sample. The assay was tested using three different Rnases (1:50 T1, 1:100 A/T1 and 1:1000 A/T1). Optimal banding was obtained with the 1:50 Rnase T1 digest. Additional lanes represent appropriate controls.

biological function. As a result cattle homozygous for the T allele express leptin with lower functionality and thus tend to deposit more fat relative to lean. These animals attempt to compensate for leptin with reduced biological activity by expressing more, hence the increased leptin mRNA abundance in adipose. This attempt at compensation is inadequate, however, as these cattle present with increased carcass fat content.

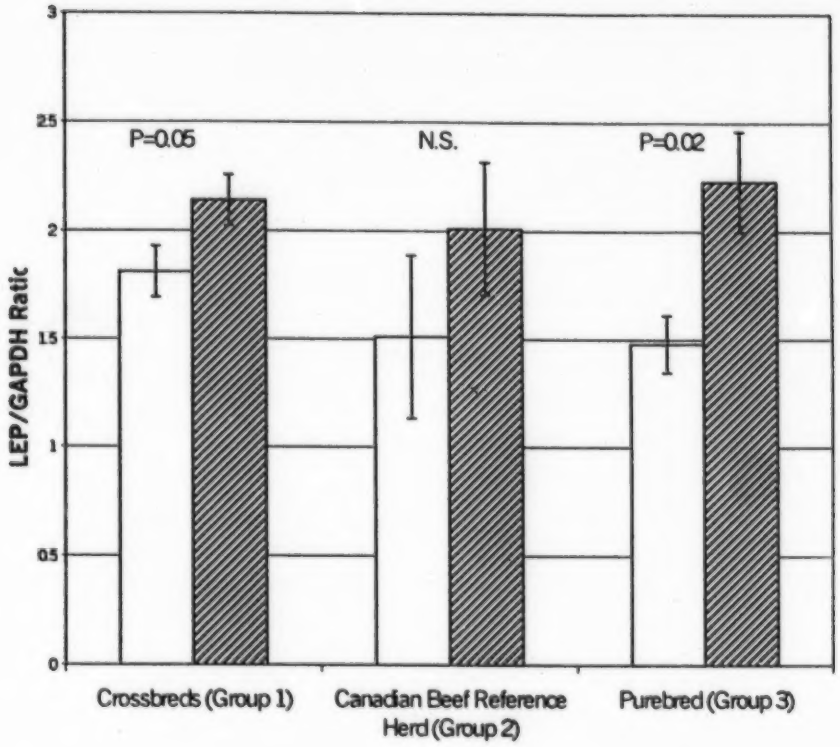


Figure 2. Relative leptin mRNA content in adipose tissue collected at slaughter in three independent groups of cattle.

Leptin Protein Quantification in Blood

Development of an assay to measure bovine leptin protein in serum has proved very difficult. While we successfully cloned and expressed significant quantities of both the wild-type and mutant full-length bovine cDNAs we could not purify significant quantities of homogenous gene product to warrant generating monoclonal antibodies. Several laboratories around the world have attempted to develop these assays and have reported similar difficulties in leptin purification. In addition, in laboratories where polyclonal antibodies have been generated, validation of bovine or swine assays using these antibodies has proven difficult. There is some consensus that serum contains substances (possibly binding proteins) that interfere with antibody-based assays. To date there are 2-3 validated leptin assays suitable for bovine available world-wide. Fortunately, we collaborated with a group at Cornell University in assay development through sharing of reagents. The Cornell group lead by Dr. Yves Boisclair successfully developed a

bovine/ovine leptin radioimmunoassay. We have utilized this assay to evaluate serum leptin levels in cattle selected on genotype at the leptin SNP as described below.

B. *In vivo* cattle experiments

Experiment 1: Interaction among Finishing Level, Breed and Genotype

This experiment employed cattle of three breed-types (Charolais, Angus and Hereford) as assessed by physical appearance (i.e. commercial non-purebreds). The cattle were backgrounded to achieve 1 kg/d bodyweight gain for a period of 70 days and finished on *ad libitum* feed intake to either 6 or 12 mm backfat monitored using regular real-time ultrasound measurements. Blood and fat samples were collected at slaughter. Cattle were genotyped at the leptin SNP using a purposeful mismatch restriction fragment length polymorphism (RFLP) test developed as part of this project. The original gene test for the leptin SNP relied upon an enzyme known to be inhibited by PCR reagents and hence required a purification step prior to digestion. The new test provided a more reliable, faster and simpler assessment of leptin genotype and was used to select 96 of the 264 head in the trial. These 96 animals were either homozygous for the T allele (T) or the C allele (CC). Heterozygotes were excluded from the study. Using a 3X2 factorial design we examined the impact of leptin genotype, including interactions with breed type and endpoint backfat thickness, on performance parameters, carcass quality and endocrine regulators of nutrient metabolism.

Results of this study are summarized in Table 1. Significant effects of leptin genotype were observed for total number of days on feed and for final live weight and hot carcass weight. Cattle homozygous for the TT allele reached target backfat depth approximately 14 days sooner than their CC counterparts (180.6±4.7 vs. 195.2±5.0 d). In addition, TT cattle reached target backfat depths at lighter final body weights (550.9±7.5 vs. 588.0±7.9 kg). These observations are important in that they confirm an association between leptin genotype and rate of fat deposition in beef cattle. In this case, since animal were fed to a backfat depth endpoint, the impact of the T allele was observed as reduced days on feed to reach the target finishing level. In addition, since TT cattle reached target finishing level at lighter body weights, the propensity of animals with the T allele to deposit fat at the expense of lean tissue was observed.

To determine if the association between the T allele at the leptin SNP and the propensity for increased fat deposition could be considered a causative SNP we measured serum leptin and insulin levels prior to slaughter. Insulin is a primary regulator of leptin secretion, however, no effect of leptin genotype on serum insulin was observed. A breed by backfat endpoint by genotype interaction was observed for serum leptin concentration at slaughter. Further analysis indicated that genotype significantly affected leptin concentration in the 12 mm Charolais crossbred group only. Homozygous TT Charolais crossbreds had significantly higher plasma leptin versus homozygous CC Charolais

crossbreds. Serum leptin concentration was higher, but not significantly, in TT Angus crossbreds at both backfat depths and in TT Hereford crossbreds in the 6 mm backfat group. The results suggested, although not strongly, that leptin genotype could impact leptin expression levels supporting the identified polymorphism as a causative SNP.

Preliminary data from this study was presented in July 1999 at the annual Meeting of the American Society of Animal Science and is being prepared as a manuscript for publication in the American Journal of Animal Science.

Table 1. Effect of genotype at the leptin SNP on serum hormone concentrations, performance and carcass traits.

	Angus				Charolais				Hereford				
	6 mm		12 mm		6 mm		12 mm		6 mm		12 mm		
Item	TT	CC	TT	CC	TT	CC	TT	CC	TT	CC	TT	CC	Significance[a]
Serum hormone concentration, ng/mL													
Leptin	7.1	5.8	10.5	9.1	6.4	7.5	13.1[b]	9.7[c]	9.7	8.6	9.5	10.8	BRxEPxGN
Insulin	12.1	20.4	21.5	27.4	11.2	17.7	19.5	16.6	15.8	19.6	18.0	12.6	
Days on feed													
Backgrounding	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	
Finishing	73.4	88.0	114.7	120.3	92.0	109.9	174.4	193.8	79.6	90.7	128.0	140.0	BRxEP, GN
Total	143.4	158.0	184.7	190.3	162.0	179.9	244.4	263.8	149.6	160.7	198.0	210.0	BRxEP, GN
Weight gain, kg													
Backgrounding	70.1	67.5	58.1	68.9	69.2	79.2	67.6	70.5	62.4	72.4	66.4	62.4	BRxEPxGN
Finishing	151.3	166.0	212.1	243.9	156.1	220.2	291.8	323.0	151.5	189.4	240.8	246.6	BR, EP, GN
Total	221.4	233.5	270.3	312.8	225.3	299.3	359.4	393.5	213.9	261.8	307.2	309.0	BR, EP,GN
Average daily gain, kg													
Backgrounding	1.00	0.96	0.83	0.98	0.99	1.13	0.97	1.01	0.89	1.03	0.95	0.89	BRxEPxGN
Finshing	2.04	1.88	1.87	2.09	1.70	2.01	1.73	1.70	1.95	2.11	1.90	1.80	BRxEPxGN
Overall	1.54	1.46	1.47	1.66	1.38	1.67	1.50	1.51	1.44	1.63	1.55	1.47	BRxEPxGN
Final weight, kg													
Live weight	521.4	532.1	570.8	622.0	529.0	601.8	671.4	707.2	527.6	565.4	615.8	626.7	BR, EP, GN
Hot carcass weight	286.0	295.5	319.4	350.5	293.9	334.8	374.8	402.1	290.9	304.9	335.2	354.0	BR, EP, GN
Carcass Grade													
Mean fat, mm	7.6	7.2	12.5	11.8	7.3	8.3	11.0	12.4	9.5	6.6	11.8	12.9	EP
Grade fat, mm	6.1	7.2	11.4	11.1	6.1	7.1	9.7	11.1	7.4	5.7	10.6	12.3	EP
Grade ribeye area	79.9	80.6	79.3	85.9	86.5	88.1	90.4	93.8	79.0	80.5	77.5	82.7	BR
Marbling score	1.6	2.0	2.0	2.1	1.3	1.9	2.6	2.9	1.4	1.2	1.9	2.2	BR, EP

[a]Significant ($P < 0.05$)main effects of breed (BR), ultrasound endpoint (EP) and leptin genotype (GN) or their interactions are indicated.
[bc]Means in the same row with different superscripts differ ($P < 0.05$)

Experiment 2: Intensive Physiological Investigation.

The objective of this study was to determine if genotype at the leptin SNP affects leptin secretion in response to challenge. Thirty unrelated purebred Simmental steers were purchased in the Spring of 1999. The steers were selected based on genotype at the BM1500 microsatellite which is tightly linked to the leptin SNP. Ten steers were AA, 10 were AB and 10 were BB at the microsatellite. Later assessment of these cattle using the direct purposeful mismatch test for the leptin SNP indicated that 14 animals were CC, 8 were CT and 8 were TT. The cattle were maintained at a high level of feed intake (2.5 X maintenance energy requirement), subject to a 10 day feed restriction (1.5 X maintenance requirement) period and then returned in a stepwise process to full feed (2.5 X maintenance). Blood samples were obtained at regular intervals over the study period. These samples were stored at –20 ^{0}C and analysed for bovine leptin and insulin concentration.

Ultrasound back fat (USFAT) data obtained 24 hours before slaughter and Blue Tag carcass data for these cattle are presented in Table 2. Comparison of ultrasound and carcass data indicate no significant effect of leptin genotype. Whether an effect would be detectable in this small group of steers is debatable, as they were fed to a common level of finish which would eliminate any genotypic differences.

Figures 3 and 4 illustrate the serum leptin and insulin profiles, respectively, during full feeding and feed restricted periods. A clear impact of feed restriction was observed such that levels of both hormones declined acutely and significantly during feed restriction and returning to elevated levels during refeeding. The results confirm a strong association between serum leptin levels in cattle and energy balance as observed for monogastric species. The decline in leptin during feed restriction is expected to increase the drive for feed intake and possible reduce the partitioning of energy towards adipose depots. Insulin is a stimulator of leptin secretion. During feed restriction insulin levels decline reflecting reduced energy absorption. The reduction in insulin also lowers partitioning of nutrients to adipose. The lower insulin levels also indicate a reduced stimulus for leptin secretion as supported by the reduced leptin levels observed.

Genotype at the leptin SNP did not affect the serum leptin or insulin profile. Serum leptin levels tended to be higher in cattle with one (CT) or two (TT) T alleles, however this was not significant. The results of this Simmental study do not provide strong support for the suggestion that the leptin SNP is a causative SNP responsible for inducing physiological changes leading to an increased propensity for fat deposition.

C. *In vivo* Bioassay of bovine Leptin activity.

The objective of this study is to determine whether the amino acid change in the leptin protein caused by the C to T nucleotide transition results in a partial loss of biological function. We hoped to establish that the leptin synthesized in cattle with the TT genotype is less functional than wild-type leptin (from CC cattle). This information would be

Table 1. Ultrasound backfat (USFAT) and Blue Tag Carcass data for Simmmental steers selected for genotype at the leptin locus

Kill Date	Pen #	Tag #	SNP Genotype C=lean T=fat	BM 1500 Genotype B=lean A=fat	Average USFAT mm	Ship Weight (kgs)	Shrunk Ship Wt (kgs)	Shrunk Ship Wt (lbs)	Hot Carcass Wt (lbs)	Dressing Percent %	Average Fat mm	Grade Fat mm	Grader REA cm^2	Grader Marbling Score	Ruler Yield %
15-Sep-99	2	513	CC	BB	8.3	710.0	688.7	1518.6	869.5	57.3	8.3	6	98	3	64
29-Jul-99	3	514	CC	BB	7.0	760.0	737.2	1625.5	901.0	55.4	10.0	6	90	4	63
29-Jul-99	3	517	CC	BB	6.5	785.5	761.9	1680.1	994.0	59.2	8.3	6	110	2	64
15-Sep-99	2	518	CC	AB	6.5	616.5	598.0	1318.6	781.5	59.3	4.7	4	97	3	62
29-Jul-99	3	519	CC	AB	8.0	730.5	708.6	1562.4	921.5	59.0	9.0	5	99	4	64
29-Jul-99	3	520	CC	BB	10.5	774.0	750.8	1655.5	926.5	56.0	10.3	8	91	2	61
15-Sep-99	2	523	CC	BB	8.8	681.5	661.1	1457.6	847.0	58.1	8.3	6	93	3	61
15-Sep-99	2	524	CC	BB	7.8	750.5	728.0	1605.2	911.0	56.8	5.3	5	99	4	61
15-Sep-99	2	535	CC	BB	8.5	691.5	670.8	1479.0	788.0	53.3	5.7	5	89	3	61
29-Jul-99	1	537	CC	BB	6.0	596.0	580.1	1279.0	739.0	57.8	10.0	7	98	2	62
15-Sep-99	2	538	CC	AA	6.8	746.0	723.6	1595.6	922.5	57.8	9.0	6	96	4	61
29-Jul-99	1	539	CC	BB	11.0	667.0	647.0	1426.6	836.5	58.6	10.7	9	93	3	59
15-Sep-99	2	541	CC	BB	7.8	715.0	693.6	1529.3	880.0	57.5	8.0	7	101	3	64
29-Jul-99	1	543	CC	AB	7.8	689.5	668.8	1474.7	813.5	55.2	10.7	6	98	2	62
					8	706.3	687.0	1514.8	866.5	57.2	8	6	97	3	62
					0.4	14.9	14.4	31.8	18.6	0.5	0.5	0.3	1.4	0.2	0.4
29-Jul-99	1	515	CT	AB	12.5	821.0	796.4	1756.0	1001.5	57.0	11.0	10	103	3	59
15-Sep-99	2	516	CT	AB	8.3	724.5	702.8	1549.6	915.0	59.0	7.3	5	101	4	61
15-Sep-99	2	522	CT	AB	5.0	676.0	655.7	1445.9	819.5	56.7	4.3	4	101	4	64
29-Jul-99	3	521	CT	AA	9.0	808.0	783.8	1728.2	1004.5	58.1	7.0	5	99	2	64
29-Jul-99	3	526	CT	AB	10.5	689.5	668.8	1474.7	843.0	57.2	12.0	8	98	3	62
29-Jul-99	1	529	CT	AB	8.5	742.0	719.7	1587.0	933.0	58.8	11.7	8	101	4	61
15-Sep-99	2	531	CT	AB	9.0	790.5	766.8	1690.8	1002.0	59.3	12.0	9	107	2	61
15-Sep-99	2	532	CT	AB	8.8	733.0	711.0	1567.8	.	.	.	.	.	.	.
					9	748.1	725.6	1600.0	931.2	58.0	9.3	7.0	101	3	62
					0.7	19.0	18.4	40.6	29.2	0.4	1.2	0.9	1.1	0.3	0.7
15-Sep-99	2	525	TT	AA	7.5	692.0	671.2	1480.1	844.0	57.0	5.7	4	103	2	62
29-Jul-99	1	527	TT	AA	7.5	634.5	615.5	1357.1	821.0	60.5	5.3	4	97	2	64
15-Sep-99	2	528	TT	AA	7.3	750.5	728.0	1605.2	907.0	56.5	6.0	5	100	4	64
29-Jul-99	3	530	TT	AA	8.8	818.5	793.9	1750.6	967.0	56.4	9.7	9	110	3	61
15-Sep-99	2	533	TT	AA	8.3	668.0	648.0	1428.8	815.5	57.1	8.7	5	96	4	64
29-Jul-99	1	534	TT	AA	8.5	663.0	643.1	1418.1	854.5	60.3	6.7	7	109	2	62
15-Sep-99	2	540	TT	AA	6.8	756.5	733.8	1618.0	935.0	57.8	5.3	4	114	2	61
29-Jul-99	1	542	TT	AA	11.0	730.5	708.6	1562.4	906.5	58.0	11.3	8	104	3	62
					8	714.2	692.8	1527.5	883.8	57.9	7.3	5.8	104	3	63
					0.5	21.5	20.8	46.0	21.3	0.6	0.8	0.7	2.3	0.3	0.5

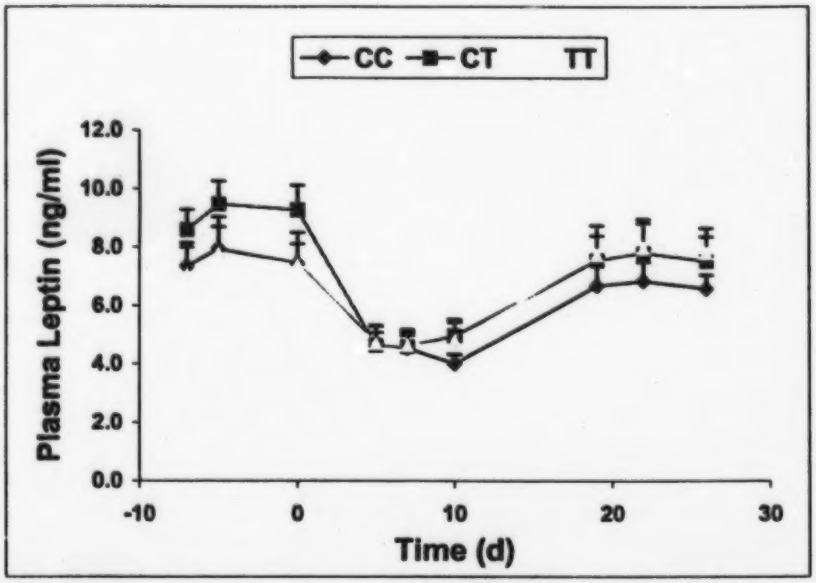
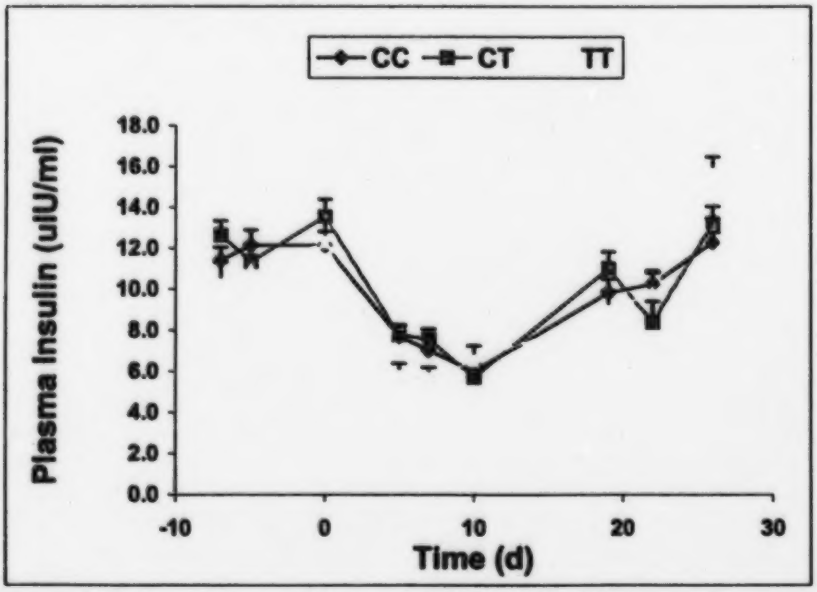


Figure 3. Serum leptin profile in unrelated purebred Simmental steers selected on genotype at the leptin SNP. Steers were moved from full feed (2.5X maintenance energy requirements) to feed restriction (1.5X maintenance) from day 0 to day 10 returning to stepwise to full feed at day 20.



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highly significant in determining whether selection of cattle based on this mutation is warranted.

Often, assays assessing biological function are performed *in vitro* using cell culture. However, in the case of leptin, there is currently no culturable cell type for which a biological response can be linked directly to leptin interaction with its surface membrane receptor. In this case an *in vivo* assay of biological function was required. We proposed to use the sheep as a model animal to show the biological potency of the two forms of bovine leptin. The sheep is well suited to this task as it is a small inexpensive ruminant animal with strong sequence homology to cattle.

We proposed to use gene therapy to deliver wild type (normal) and mutated leptin to lambs. We have experience in this technology in pigs and have measured the appearance of a reporter gene in serum of pigs subject to a gene therapy treatment. In the current proposal we intended to deliver one of two plasmids into the leg muscle of lambs. One plasmid would direct synthesis of leptin with the C allele and the other the T allele. Each plasmid would be administered in each of 10 lambs. An additional 10 lambs would function as an untreated control group. Plasmid administration will be followed by *in vivo* electroporation of the site to enhance plasmid uptake and level of expression. Feed intake, ultrasound recorded backfat accumulation, carcass evaluation and fat leptin mRNA level were to be recorded. We cloned the two forms of the leptin gene into a mammalian expression plasmid suitable for *in vivo* delivery in lambs. Next we attempted to generate large quantities of the plasmid (40 mg) required for injection. Unfortunately, multiple attempts to generate large quantities o f these plasmids failed. This plasmid containing a different gene was than used previously for our pig studies yielding large quantities. However, with the leptin gene present we were unable to generate significant plasmid yield (<1 mg/10 L fermentation) on extraction from bacterial culture. Numerous different clones of these plasmids were tested in several different laboratory *Escherichia coli* strains. Invariably, yields of plasmid per litre of bacterial culture were very low Large scale plasmid preparation would have required unworkably large volumes of bacterial culture and extremely high costs for purification reagents.

We had delayed reporting on this study in order to complete this important *in vivo* study to assess biological activity of the two leptin forms. Unfortunately, these technical difficulties have prevented completion of this portion of the project. As a result we do not have conclusive evidence as to the causative nature of the leptin polymorphism with respect to the propensity for increased fat deposition. Research funds dedicated to this portion of the study have been returned to the Agri-Food Innovation Fund.

Indicators for future development

We believe that a significant contribution of this project to livestock production will be through confirmation of the value of selecting cattle using the leptin SNP. We have confirmed an association between the leptin SNP and the propensity for fat deposition in steers. As a result, of these observations further industry-supported studies are currently being discussed to assess the value of the leptin SNP in large scale feed lot studies. The purposeful mismatch PCR-RFLP developed under this project will provide a valuable tool for these studies as a reliable method for determining leptin genotype. It is anticipated that this work has laid the foundation for commercial utilization of a genetic test allowing segregation of cattle at feedlot entry based on the predicted propensity for fat deposition. Cattle segregated based on the leptin SNP can be assigned to tailored feeding programs and target market markets to provide for maximum feedlot profitability.

Deliverables and performance indicators

We had proposed development of two methods for analysis of leptin physiology including analysis of mRNA and RIA/ELISA analysis of serum leptin. We have developed a sensitive ribonuclease protection assay (RPA) to quantify leptin mRNA levels in fat tissue. This assay has provided the most convincing evidence that the leptin SNP is causative with respect to fat deposition rate. We have collaborated with a research group at Cornell University to obtain access to an assay for measurement of leptin in bovine serum. We have used this assay in our investigation, however, the results of serum leptin analysis do not provide strong support for the leptin SNP as the causative SNP. Through our collaboration with Cornell we will be able to examine serum leptin levels in future large feedlot studies in an attempt to understand more fully the relationship between the SNP, serum leptin concentration and breed type. As an example, The serum leptin analysis suggests that the leptin SNP may have the greatest effect on fat deposition in the Charolais breed.

c) Personnel

Kimberly Bryce was hired as the summer student in 1997.
Bobbi Jo Prosofsky was hired as a summer student in 1998.
Dr. David Ekpe was hired as a postdoctoral fellow for a 1 year term.
Mika Asai was hired as the summer student in 1999.

d) Equipment

1997

Techne Genius PCR machine (serial # 73526)
Pharmacia powerpack (serial # 56115552 G9 004252)
Sequencing gel electrophoresis apparatus (serial # 20015909))
1998
1999
2000

e) Project Photos

N/A

f) Innovation Fund Acknowledgment

The Star Phoenix interviewed Drs. Andrew van Kessel and Fiona Buchanan - Research aimed at leaner beef, August 20th 1997 (attached)

g) Project Public Information Materials

Van Kessel, A.G., Buchanan, F.C., Block, H.C., McKinnon, J.J., Laarveld, B. and Schmutz, S.M.. 2002. A leptin single nucleotide polymorphism affects performance, carcass traits and plasma leptin concentration at slaughter determined in cattle. Journal of Animal Science (in preparation).

F.C. Buchanan, C.J. Fitzsimmons, A.G. Van Kessel, T.D. Thue, D.C. Winkelman-Sim and S.M. Schmutz, 2002. Association of a missense mutation in the bovine leptin gene with carcass fat contents and leptin mRNA. Molecular Genetics Evolution, 43, 1-12.

F.C. Buchanan, 2002. Effects of a bovine leptin missense mutation on animal performance. Plant, Animal and Microbe Genome X, January, San Diego, CA. (invited talk).

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Van Kessel, A.G., Buchanan, F.C., Block, H.C., McKinnon, J.J., Schmutz, S.M. and Laarveld, B. (1999) Relationship among genotype at the BM1500 microsatellite, performance and carcass traits and plasma leptin concentration at slaughter determined by a multi-species RIA in cattle. Journal of Animal Science, 77 (Suppl. 1): 228.

Van Kessel, A.C., C.J. Fitzsimmons, C.J., Buchanan, F.C., Laarveld, B. and Schmutz, S.M. Mutation in the leptin gene affects carcass quality and performance in cattle. Proceedings of the 3rd National Beef Science Seminar: Molecular Biology in the Service of the Beef Industry. August 20, 1999, Edmonton, AB.

Van Kessel, A. Why are some cattle harder to finish than others – is it genetics? Proceeding of the 5th Annual Saskatchewn Beef Symposium. November 16, 1999, Saskatoon, SK.